

Adapter-directed display systems

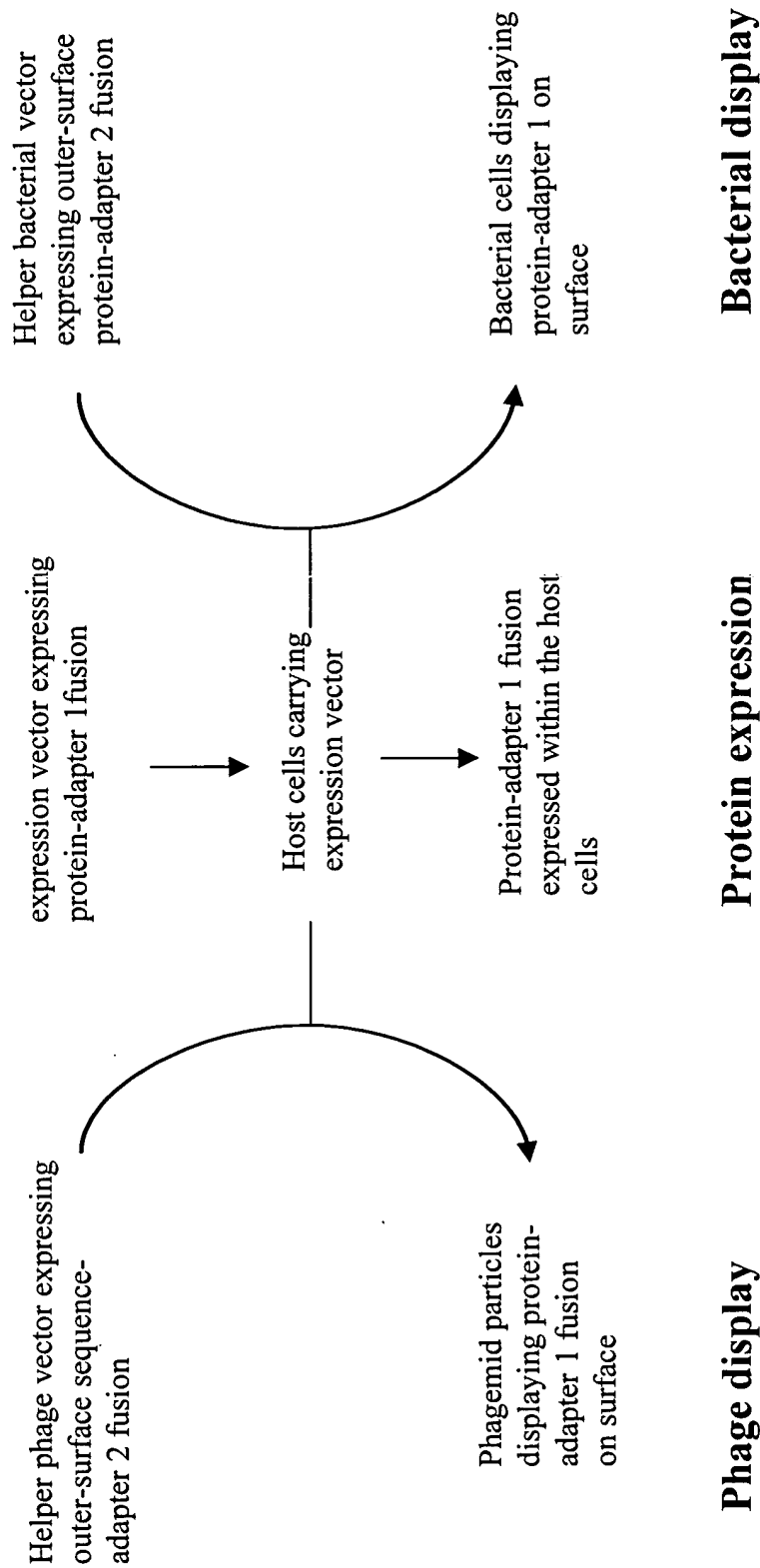


Fig. 1

KO7kpn phage Screening by ELISA

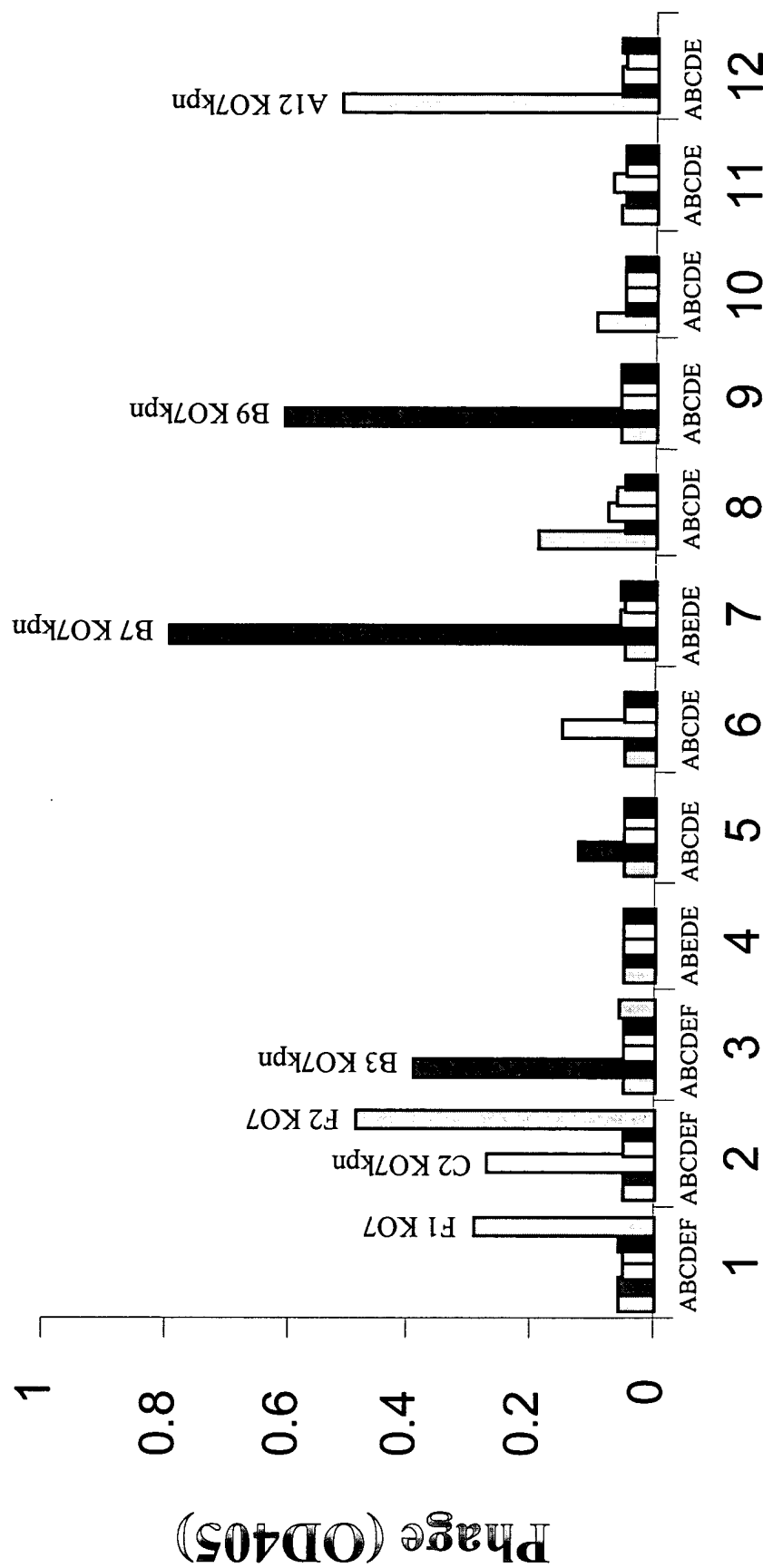


Fig. 2

KO7kpn helper phage Vector

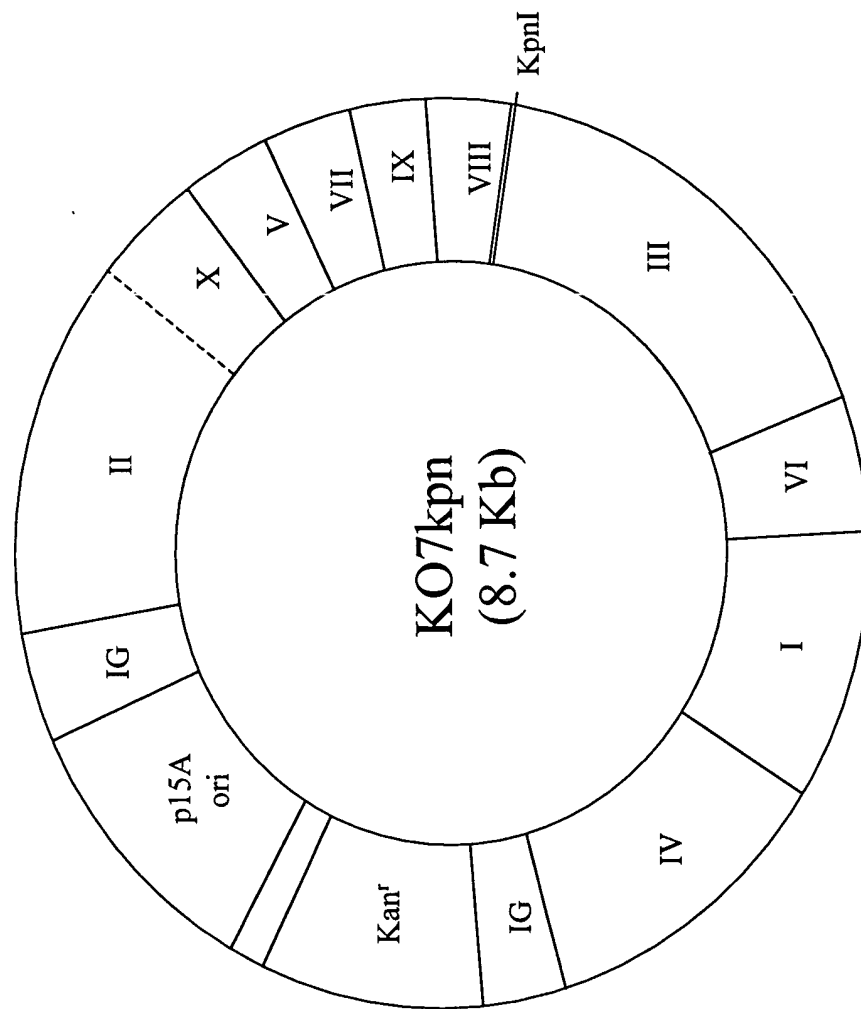


Fig. 3A

TCCTCTT" 5555555555

Gene III leader sequence in KO7 helper phage

GTG AAA AAA TTA TTA TTC GCA ATT CCT TTA GTT GTT CCT TTC TAT TCT CAC TCC GCT
V K K L L L F A I P L V V P F Y S H S A

Gene III leader sequence in KO7kpn helper phage

GTG AAA AAA TTA TTA TTC GCA ATT CCT TTA GTG GTA CCT TTC TAT TCT CAC TCC GCT
V K K L L L F A I P L V V P F Y S H S A

KpnI

Fig. 3B

Map of phagemid vector pABMC6

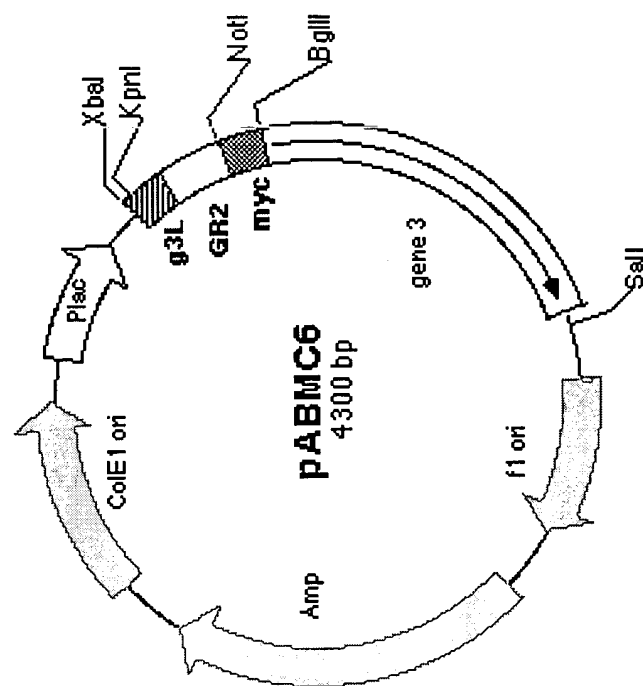


Fig. 4

Helper phage with engineered gene III fused to adaptor 2

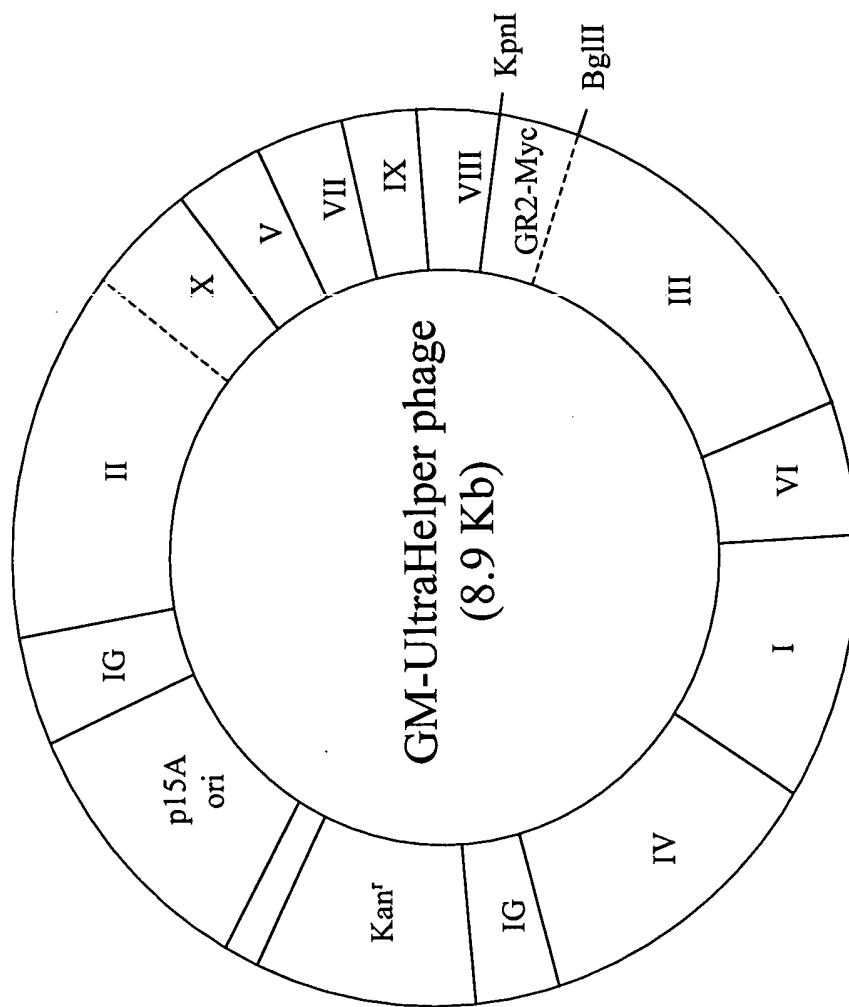


Fig. 5A

GR2-Myc domain coding sequence in GM-UltraHelper phage genome

KpnI	Gene III leader	GR2
<p>---TTAGTGGTACCTTTCTATTCTCACTCCGCT ACATCCCGCCTGGAGGGCCCTACAGTCAGAAAAACCATCGCCTGCGA</p> <p>- L V V P F Y S H S A T S R L E G L Q S E N H R L R</p>		
<p>ATGAAGATCACAGAGCTGGATAAAGACTTGGAAAGAGGTCACCATGCAGCTGCAGGACGTCGGAGGTTGC GCGGCCGCA</p> <p>M K I T E L D K D L E E V T M Q L Q D V G G C A A A</p>		
NotI		
Myc-tag	BglIII	Gene III
<p>GAACAAAACTCATCTCAGAAGAGGATCTG <u>AGATCTGGAGGCGGT</u> ACTGTTGAAAGTTGTTTAGCAAAA---</p> <p>E Q K L I S E E D L R S G G G T V E S C L A K -</p>		

Fig. 5B

TOEFT 66EEOT

Trypsin cleavage sites at GR2-Myc domain on GM-UltraHelper phage

GR2 domain

T S R L E G L Q S E N H R L R M K I T E L D K D L E E V

Myc-tag

T M Q L Q D V G G C A A E Q K L I S E E D L R S G G G

Fig. 5C

GR2-Myc-pIII fusions assembly into GM phage particles

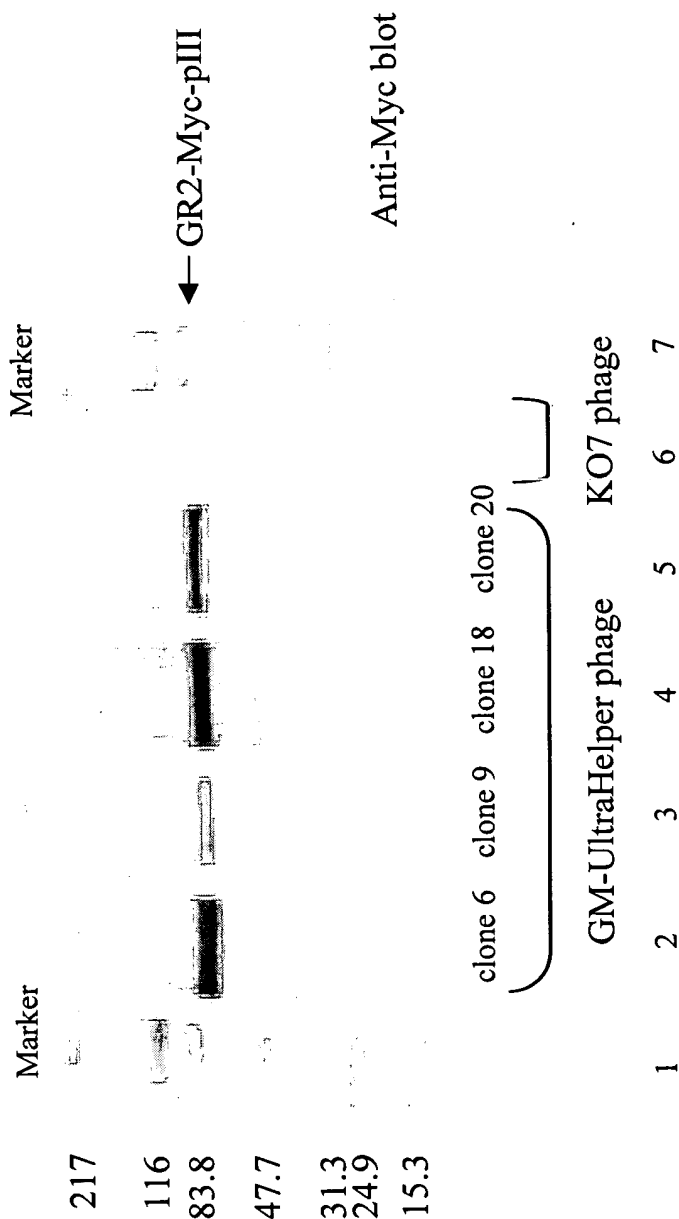


Fig. 6

Detection of GR2-Myc domain on GM-UltraHelper phage

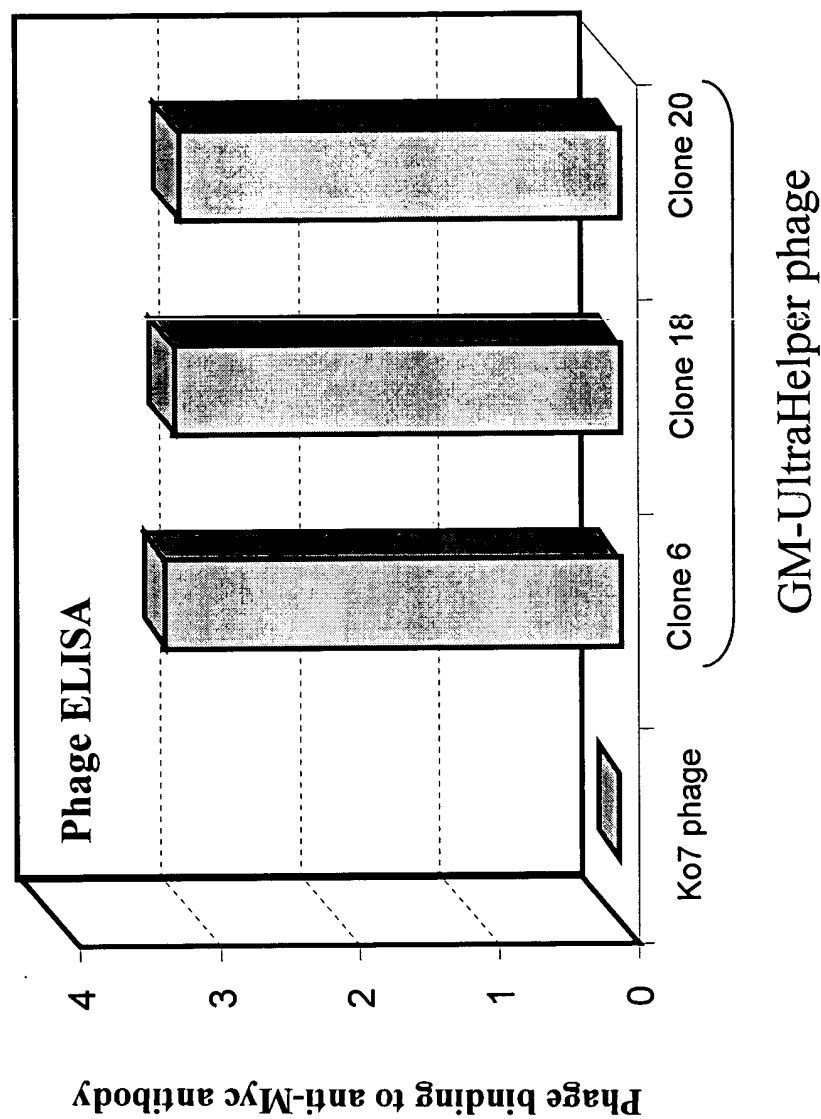


Fig. 7

Cleavage of GR2-Myc domains on GM phages by trypsin

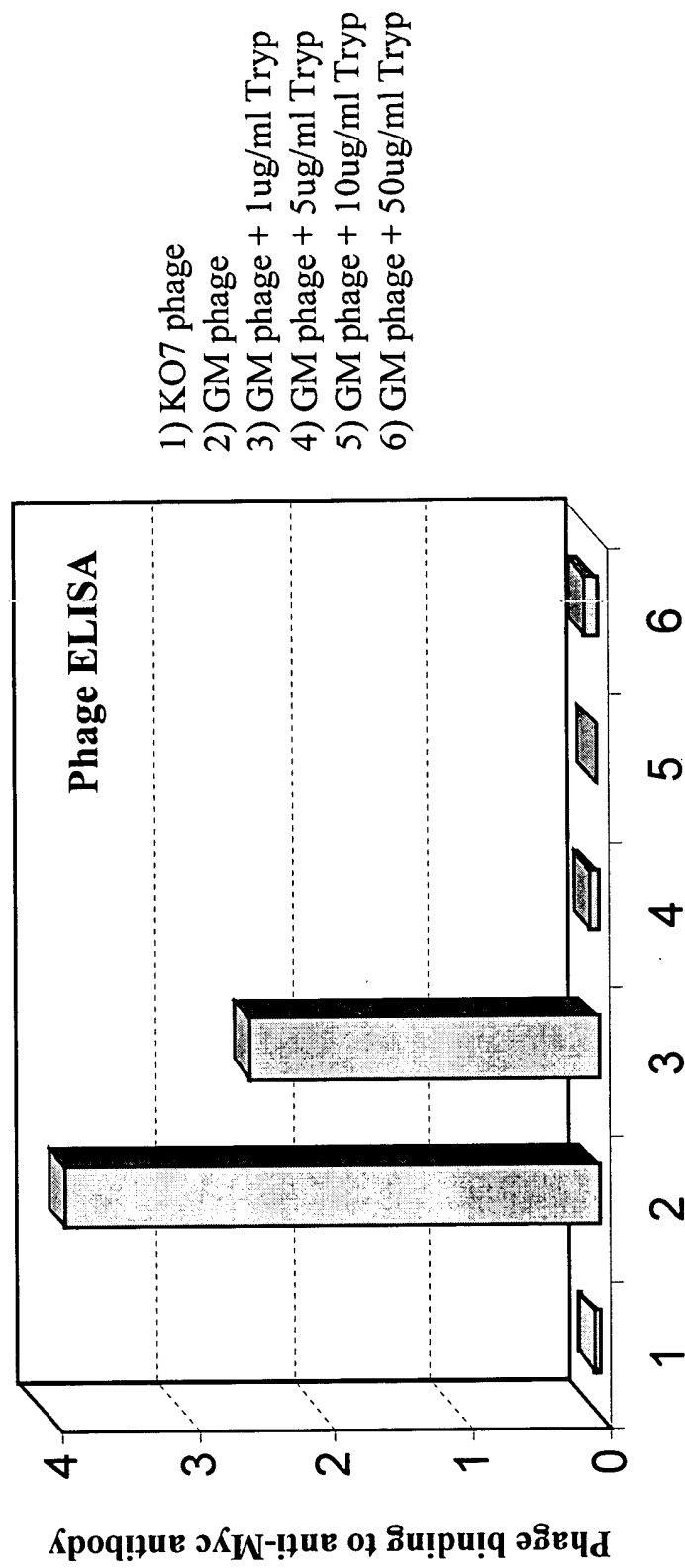


Fig. 8

Phagemid vector for protein-GR1 expression

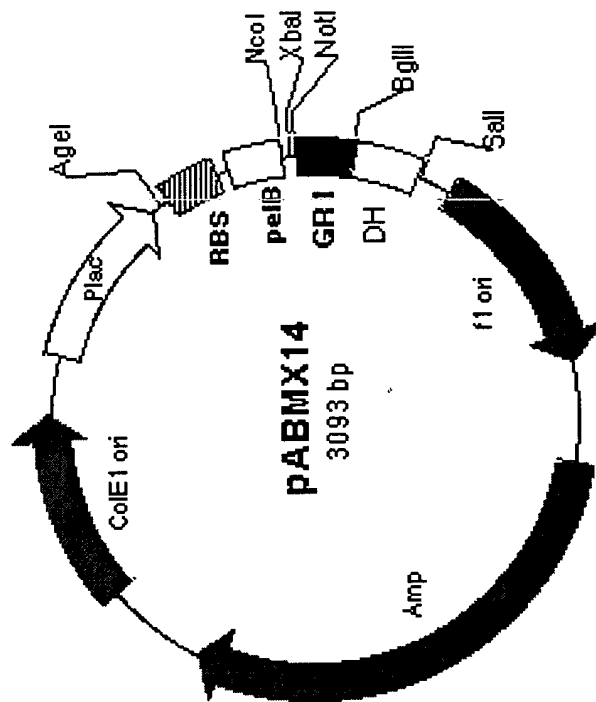


Fig. 9A

Complete vector sequence of pABMX14

GGCGCAACGCAATTAAATGTAGTTAGTCTCACTCATTTAGGCAACCCAGGCTTTACATTTATGCTTCCGGCTCGTATGTTGTGTGGAATTTGTGAGCGGATAAACAATTTACCGTCTCTTTAAGGAGGA
ATTAAAAAATGAAATACCTATTGCTACGGCAGCGGTGATTTGTTATTACTCGCGCCACAGCCGGCCATGSGGCCCCCTGCAAGSCCTTAGAGCGGCCGTGGAGGTGAGGAGAAGTCCCGGCTG
TTGGAGGAAGGAGAACCGTGAATCGAATAAGATCATTTGTAGAAAGAGAGAGGTGTCTGAACTGCGGCATCAACTCAGTCTGTAGGAGGTGTAGATCTTTATCCATACAGGAATCCACAGACTA
CCGAGGAGGTTCATCCCATCACCATCATCAAGTGTGAGCTCGACCAATTCGCCCTATAGTAGTGTCTATTAACAATTCACTGCGCTGTTTTAACAGTCTGTGACTGGGAACACCCCTGGCGTT
ACCCAACTTAATCGACACATCCCTTTCCGACGACTCGGCTGTAATAGCAGAGAGGCGCGCACCGATCGCCTTCCCAJACGACTGCGACGCTGAATGGCAATGGGACGCGCCCTGTAG
CGCGGCATTAAGCGCGCGGGTGTGTGTGTACGCGAGCTGTGAGTTCAGCCAGCTCTGAGTTCCTTCCCTTTCTTCCCTTTCTCCGACAGTTCGCGGGCTTTCCCC
GTCAAGCTCTAAATCGGGGGCTCCCTTTAGGTTCCGATTTAGTGTCTTAAGSCACCTCGACCCCAAAAACTTGATTAGGCTGATGCTTCACTGAGTGTGGCCATCGCCCTGATAGACGGTGTCTT
CGCCCTTTGACGTTGAGTTCACAGTTCCTTAATAGTGGACTCTGTTCCAAACTGGAAACAACACTCAACCCCTATCTCGGCTCTATCTTTTGTGATTAAGGATTTGCGCATTTGCGGCTATTTG
GTTAAANAATGAGTGTGATTAACAAAAATTTAAACGGAAATTTAAACGGTTCATAAATTAACGCTTCAAAATAGTGTGGCACTTTTCGGGAAATGTGCGCGGAACCCCTATTGTTTATTTCTAAAT
ACATTTCAAATATGATATCCGCTCATGAGACAATAACCCCTGATTAACGCTGTGAAGTAAAGATGCTGAAATCAGTGTGGTGTGACAGTGGGTTACATGAACTGGATCTCAACAGCGGTGAAGTCTT
GCCCTCTCTGTTTTGCTCACCCAGAAACGCTGTGAAGTAAAGATGCTGAAATCAGTGTGGTGTGACAGTGGGTTACATGAACTGGATCTCAACAGCGGTGAAGTCTTCTGAGAGTCTTCCG
CCCGAAGAACGTTTTCAATGATGAGCACTTTTAAAGTCTGCTATGTGGCGGGTATTATCCCGTATTGACCGCGGCAAGAGCAACTCGGTGCGGCCATACACTATTTCTCAGAAATGACTGTGT
TGAGTACTCACAGTCAAGAAAAAGCATTTACGAGTGCATGACAGTAAGAGATTTATGCAAGTGTGCCATTAACCATGAGTGTAAACACTCGGTGCGGCCATACACTATTTCTCAGAAATGACTGTGT
GCAAGAGACTAACCGCTTTTGTGCAAACTAATGAGGGATCATGTAATCGCTGTGATCTGTGGGAAACGGAGCTGAATGAAGCATACCAAAACGACGAGCGTGAACACCATCGATCGCTGAGCAATG
CTGTTATTTGCGCAAACTATTAACTGCGCAACTACTTACTAGCTTTCCCGCAAACTTAATAGACTGTGATGGAGCGGATAAAGTTGCAAGACACTTCTGCGTCCCTTCGCGCTG
CTGTTATTTGCTGATTAATCTTGAGCCGTCGAGCGGTGGGTCTCGCGTATCATTTGCAAGCACTGGGCGAGATGTTAAGCCCTCCCGTATCGTAGTTTACTCATATATACTTTAGATTGAATTAATAACTTCATTTTTAAATTAAGAAG
TGATGAACGGAATAGACAGATCGCTGAGTAGTGTGCCCTCACTGATTAAGCATTTGGTAACTGCTCAGACCAAGTTTACTCATATATACTTTAGATTGAATTAATAACTTCATTTTTAAATTAAGAAG
ATCTAGGTTGAAGATCTTTTGATATCTCATGCAAAATCCCTTAACGCTGAGTTTGTGTTTCACTGAGCGTACAGCCCGTGAAGAAAGATCAAAAGGATCTTCTTGAGATCTCTTTTCTCGG
CTGTTATCTGTGCTGCAAAACAAAACCGCTACAGCGGTGTGTTTGTGCGGATCAAGACTACCACTCTTTTTCAGAGGTAACTGGCTTCAGCGGCAACCAAAATCTG
TTGAGCTCAAGACGATAGTTACCGGATAAGCGACGCGTCTGAAACGGGGGTTCTGTGCAACACAGCCAGCTTGGAGCGAAACGACTTACACGAACTGAGATACCTACAGCGTGAAGTATG
AGAAAGCGCACGCTCCCGAAGGGAGAAAGCGCGACAGTATCCGTTAAGCGCAGGTCGAAACAGGAGCGGCTGTAACCGCTCGCGCAGCCAGCAACCGGCCCTTTTACGGTTCTCTGGCTTTTGTGCTCAG
GGTTTCGCACTCTGACTGAGCGTCGATTTTGTGATGCTGTCAGGGGCGCGAGCTTATGTAAGAAACGCCACAGCGGCTTTTACGGTTCTCTGGCTTTTGTGCGCTTTTGTGCTCAG
ATGTTCTTCTCGGTATCCCTGATTTCTGTGTGATAACCGTATTTACCGTTTGTAGTGAGTGTATACCGCTCGCGCAGCCAGCAACGACGCTGAGTCAAGTGTGAGCGAGCGGAAGA
GCGGCTTAATACGCAAAACCGCTCTCCCGCGGTGTGTCATTAATGCACTGGCAGCAGAGTTCCTCCGACTGGAAGAGCGGGCAGTGA

Fig. 9B

Functional display of scFv by GM-UltraHelper phage

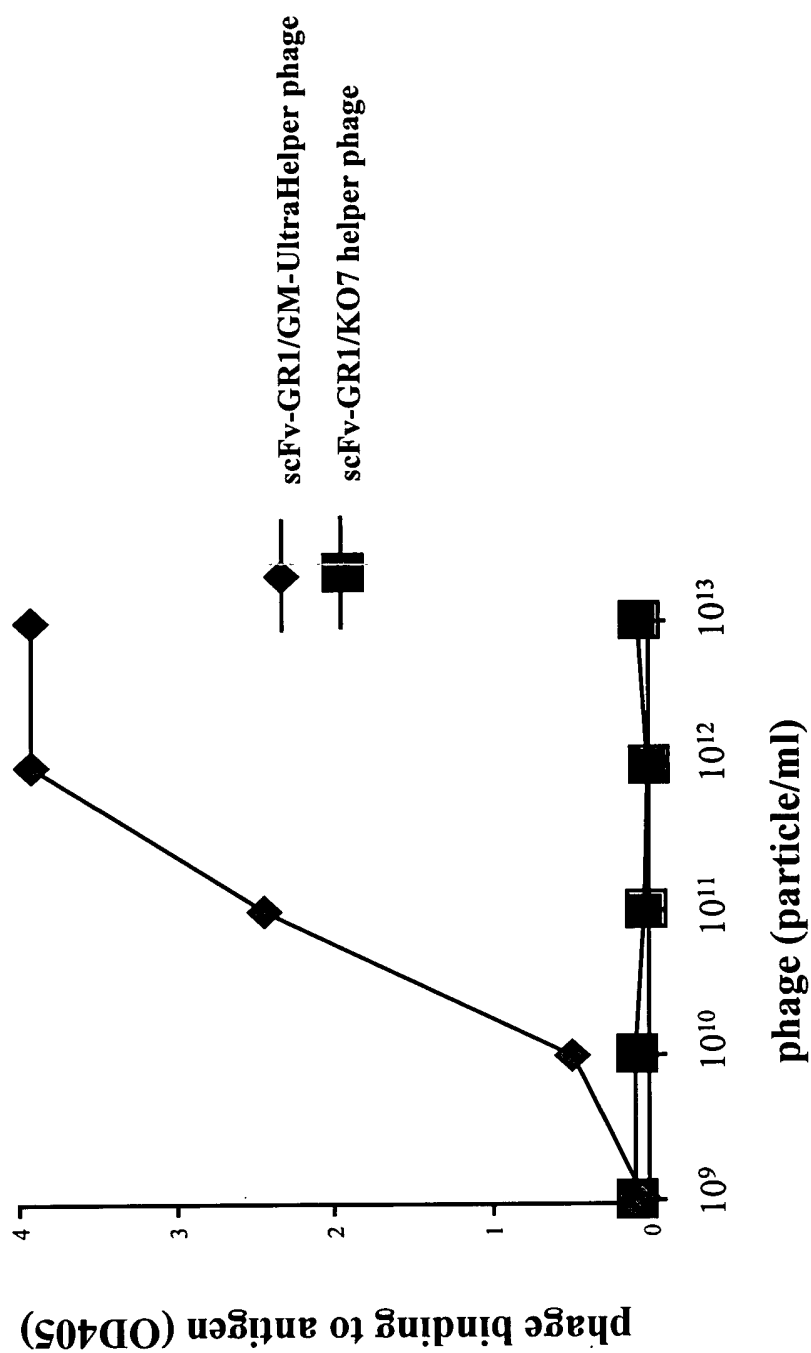


Fig. 10

Mutivalent display of scFv by GM-UltraHelper phage

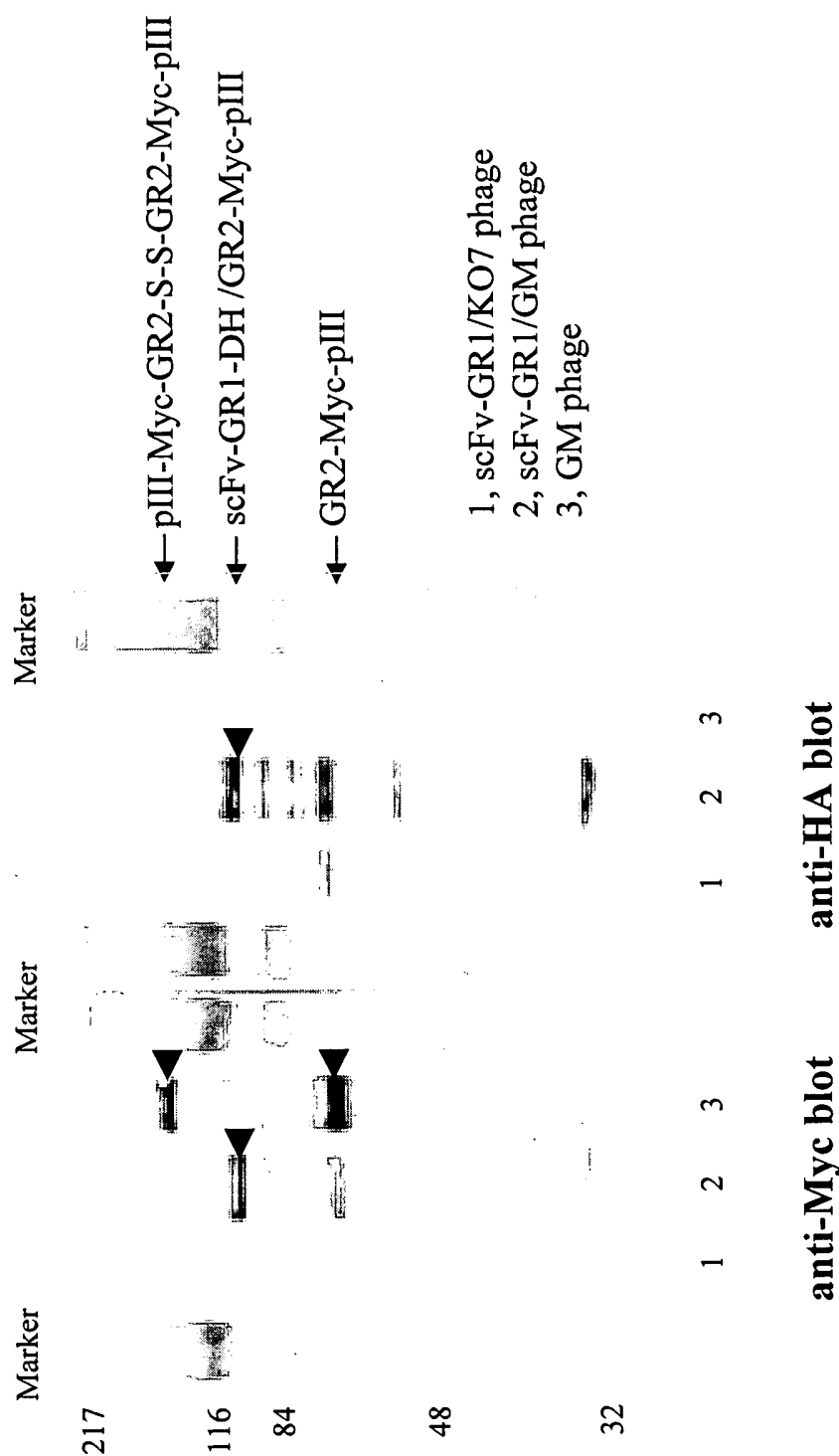


Fig. 11

Map of phagemid vector pABMC13

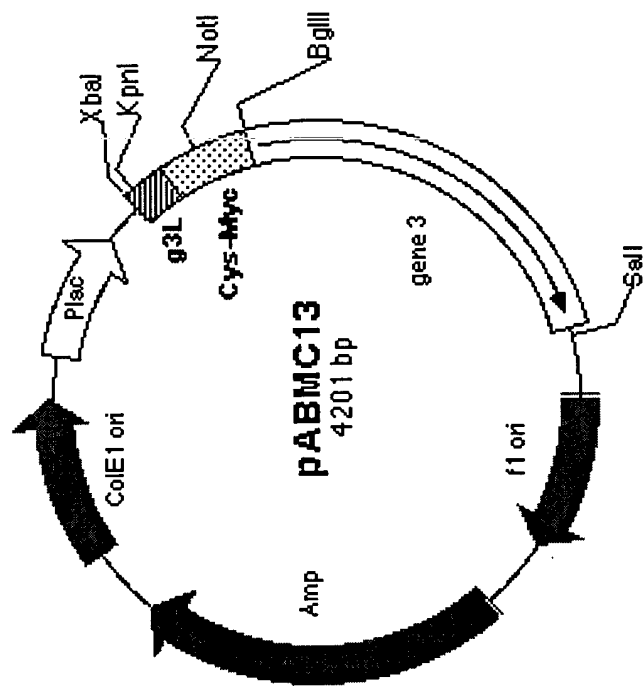


Fig. 12

Helper phage with Cys-Myc-pIII fusion gene

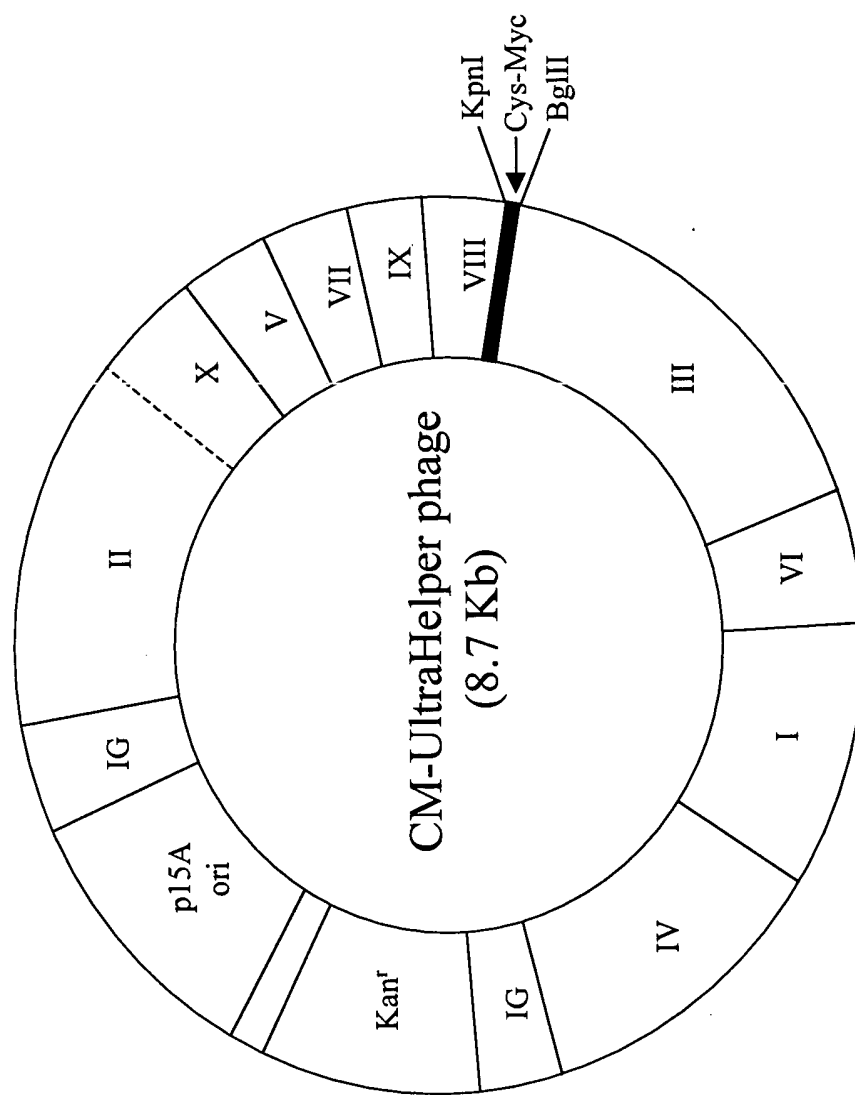


Fig. 13A

TEOTOT "SEEEDOT"

Engineered gene III sequence in CM phage

KpnI	Gene III leader	Amber stop	NotI	Myc-tag	BglII
---	<u>TTAGTGGTACCTTTCTATTCTCACTCCGCT</u>	<u>TAGGCTTGC</u>	<u>GGTGGTGGCGGCAGAACAAAACTCATCTCAGAAAGAGGATCTGAGATCT</u>	<u>AGATCTGGA</u>	
-	L V V P F Y S H S A *	A C G G A A A E Q K L I S E E D L R S R S G			

Gene III

GGCGGT	ACTGTTGAAAGTTGTTTAGCAAAACCTCATACAGAAAATTCATTACTTAACGTCTGGAAAGACGACAAAACTTTAGATCGTTACGCT	-----
G G	T V E S C L A K P H T E N S F T N V W K D K T L D R Y A	- - -

Fig. 13B

Detection of Myc-tag on CM-UltraHelper phages by ELISA

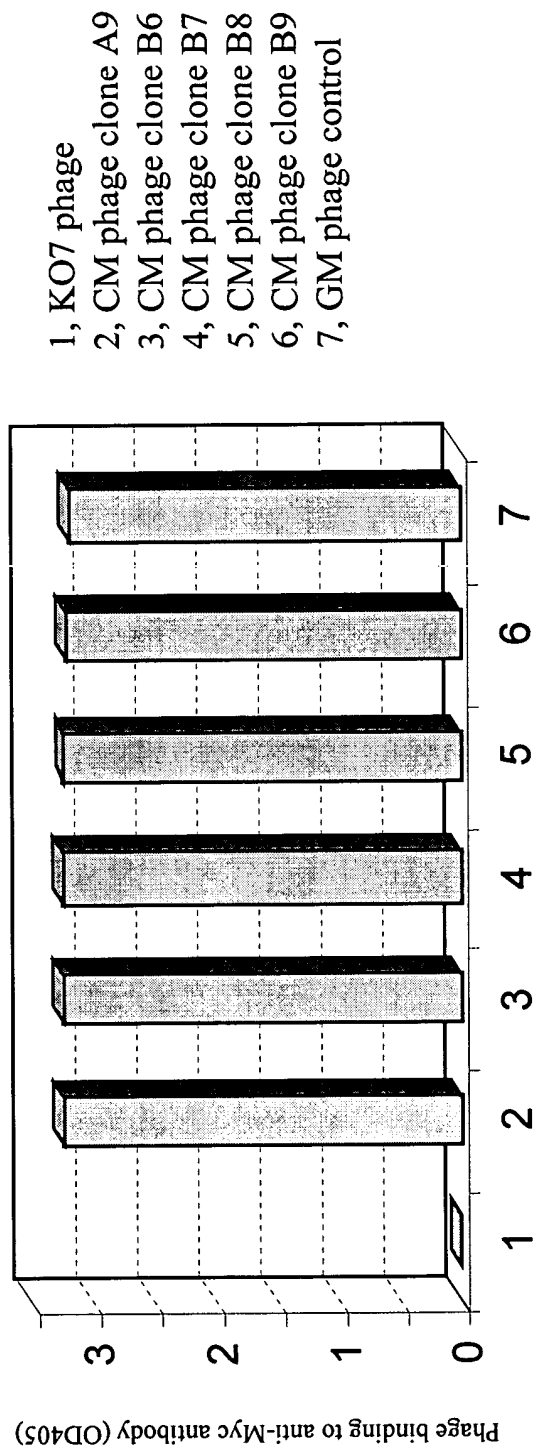


Fig. 14

Phagemid vector for protein-HA-cys expression

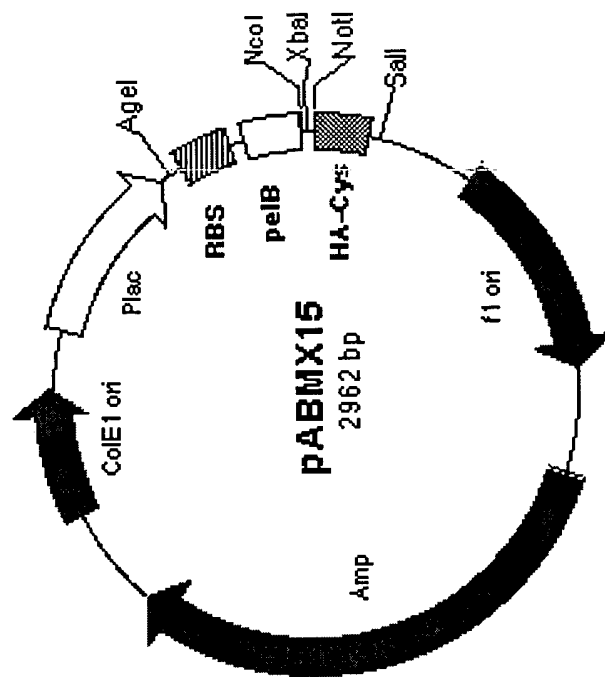


Fig. 15A

Functional display of scFv by CM-UltraHelper phage

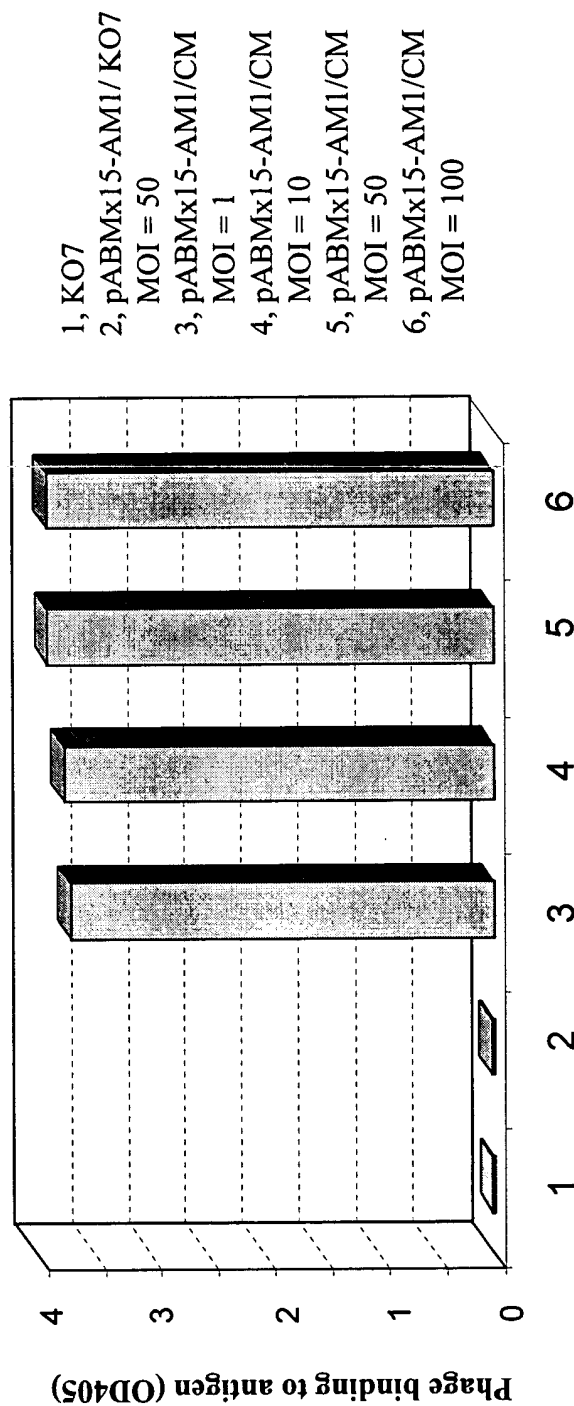
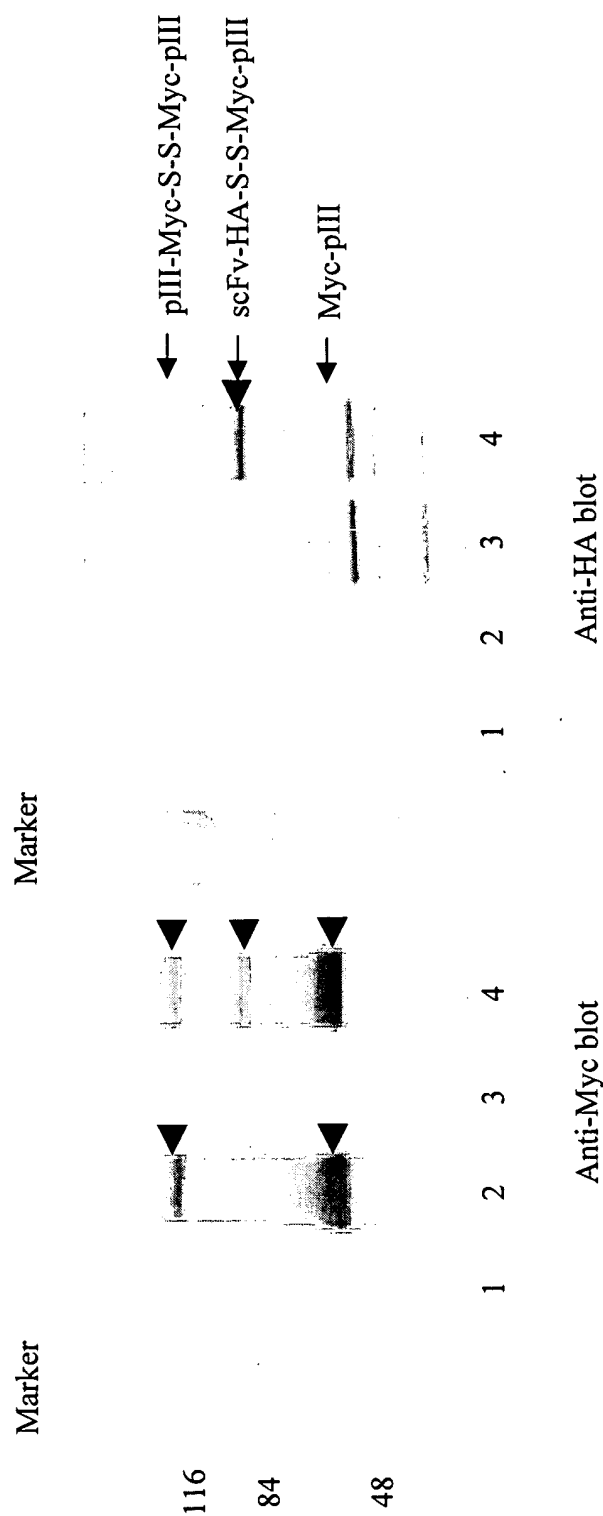


Fig. 16

Detection of scFv displayed by CM-UltraHelper phage



1: KO7 phage; 2: CM phage; 3: pABMx15-AM1/KO7; 4: pABMx15-AM1/CM

Fig. 17

Map of phagemid vector pABMC12

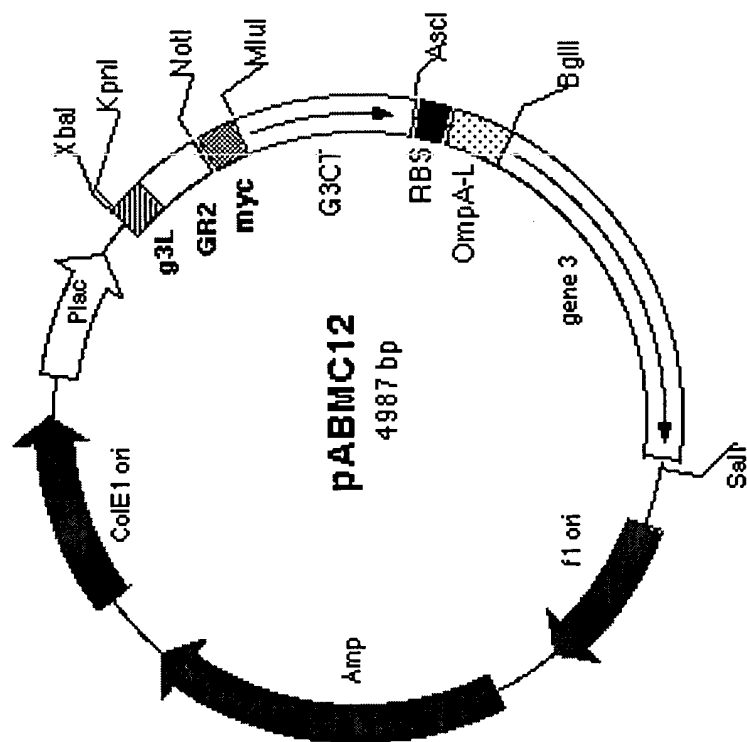


Fig. 18

Helper phage with an additional copy of engineered gene III

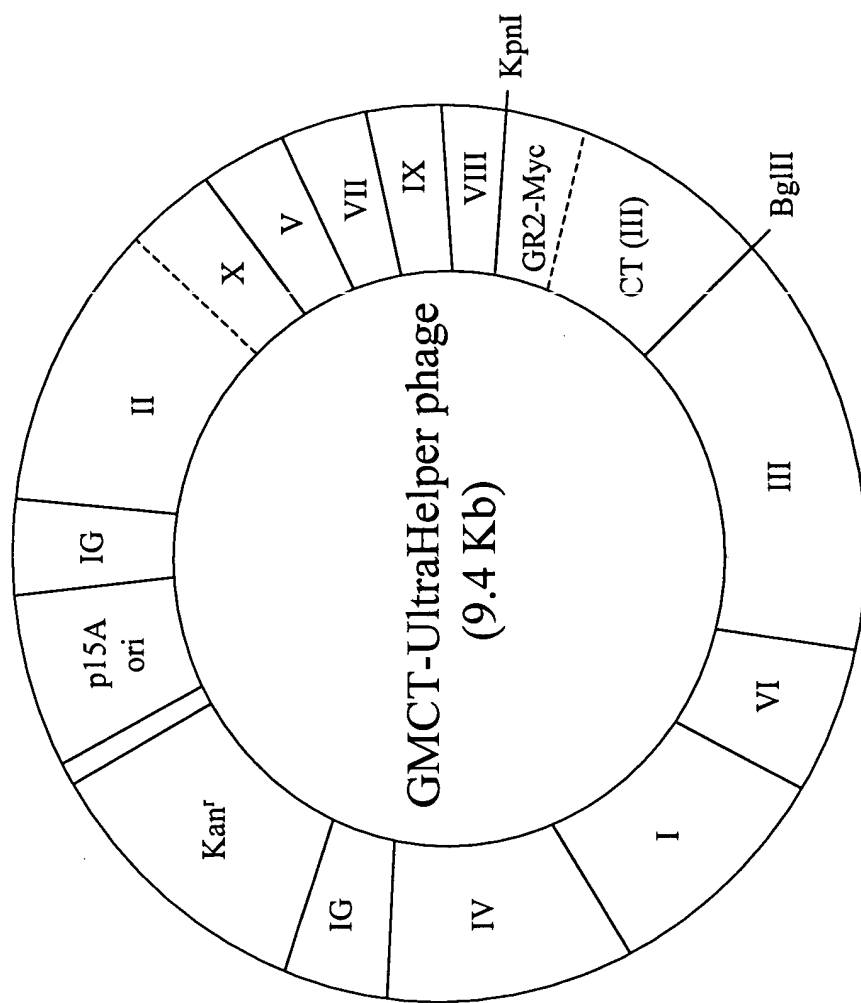


Fig. 19A

Engineered gene III Sequence in GMCT phage genome

KpnI	Gene III Leader	GR2 domain
--TTAGTGGTACCTTTCTATTCTCACTCCGCT ACATCCCGCCTGGAGGCGCTACAGTCAGAAAAACCATCGCCTGCGAATGAAGATCACAGAGCTGGATAAAA		
- L V V P F Y S H S A T S R L E G L Q S E N H R L R M K I T E L D K		Myc-tag
GACTTGAAGAGGTCACCATGCAGCTGCAGGACGTCGGAGTTGC GCGCGCGCAGAACAAAAAACTEATCTCAGAAAGAGGATCTGACGCGTGCT GGCGGC		
D L E E V T M Q L Q D V G G C A A A E Q K L I S E E D L T R A G G		NotI
CT domain of Gene III		
GGCTCTGGTGGTGTCTGGTGGCGCTCTGAGGGTGGCGGCTCTGAGGGTGGCGGTTCTGAGGGTGGCGGCTCTGAGGGTGGCGGTTCCGGTGGCGGCTCC		
G S G G G S G G S E G G S E G G S E G G S E G G S E G G S		
GGTTCGGGTGATTTTATGAAAAAATGGCAACGCTAATAAGGGGCTATGACCGAAAAATGCCGATGAAAAACGCGCTACAGTCTGACGCTAAAGGCAAA		
G S G D F D Y E K M A N A N K G A M T E N A D E N A L Q S D A K G K		
CTTGATTCTGCTACTGATTACGGTGTCTGCTATCGATGGTTTCATTGGTGACGTTTCCGGCGCTTGTCTAATGGTAATGGTGTCTACTGGTGATTTTGTGGC		
L D S V A T D Y G A A I D G F I G D V S G L A N G N G A T G D F A G		
TCTAATTCCTCCAAATGGCTCAAGTCGGTGACGGTGATAATTACCTTTAATGAATAAATTCCTGTCATAATTTACCTTCCCTCAATCGGTTGAATGTGCG		
S N S Q M A Q V G D G D N S P L M N N F R Q Y L P S L P Q S V E C R		
CCTTTGTCTTTGGCGCTGGTAAACCATATGAATTTTCTATTGATTGTGACAAAAATAAATTAATTCGGTGGTGCTTTTGGGTTCTTTTATATGTGTCACCC		
P F V F G A G K P Y E F S I D C D K I N L F R G V F A F L L Y V A T		S/D
TTTATGTATGTAATTTCTACGTTTGTCTAACATACTGCGTAATAAGGAGTCTTAATAA GCGCGCGCACAAATTCACAGTAAGGAGGTTTAATAA ATGAAA		
F M Y V F S T F A N I L R N K E S * *		AscI
OmpA leader	BglII	Gene III
AAGACAGCTATTGCGATTGCGATTGCGCTGGCTGGTTTCGCTACCGTAGCGCAGGCT AGATCTGGAGGCGGT ACTGTTGAAAGTTGTTTACAAAA--		
K T A I A I A V A L A G F A T V A Q A R S G G G T V E S C L A K -		

Fig. 19B

Functional display of scFv by GMCT-UltraHelper phage

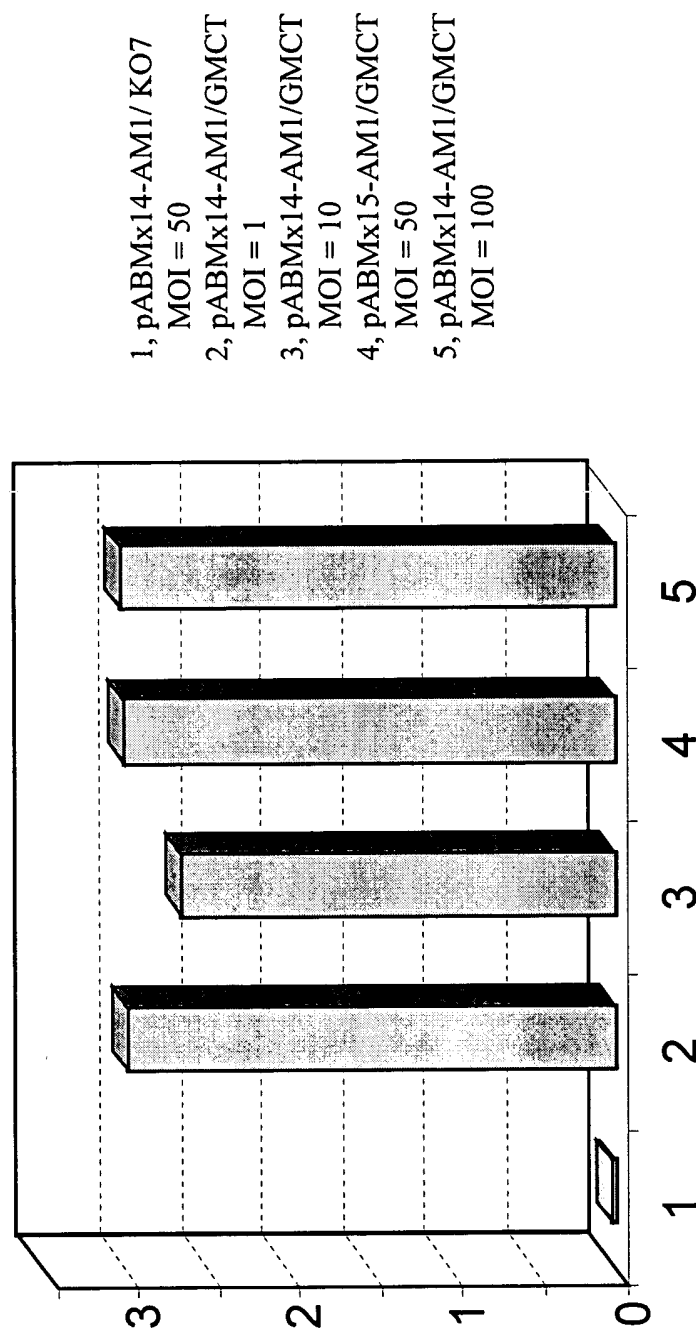


Fig. 20

Detection of scFv displayed by GMCT-UltraHelper phage

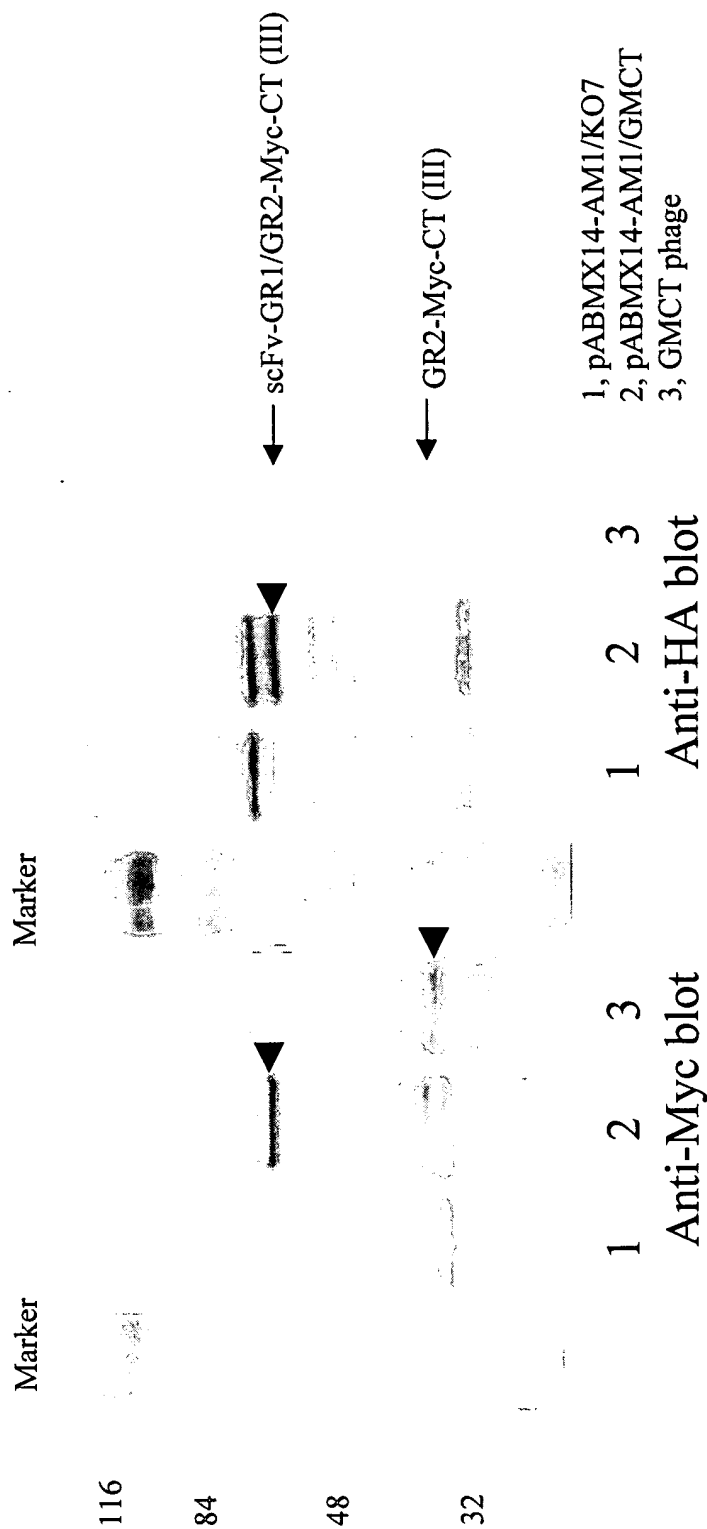


Fig. 21

2025-10-1

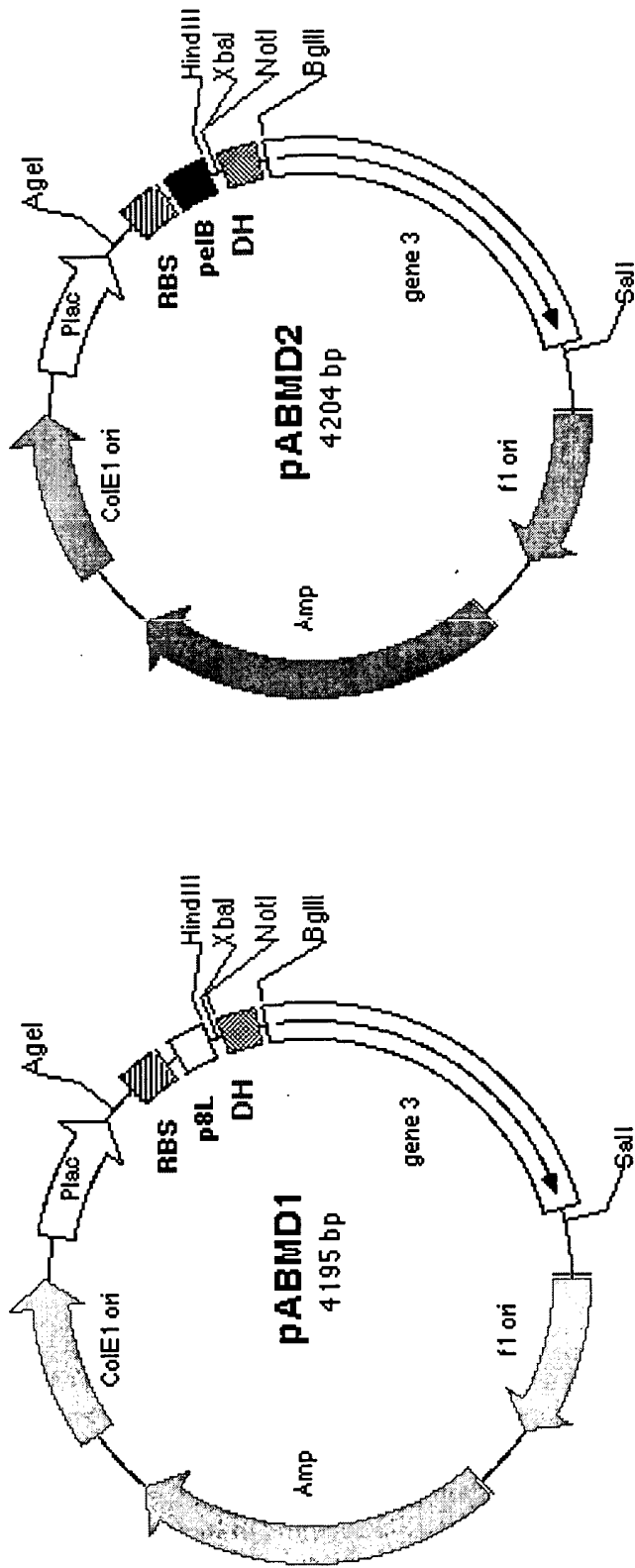


Fig. 22A

PABMD1 vector: sequence from AgeI to SalI

lac promoter/lac O1 AgeI EP S/D
AATTGTGAGCGGATAACAATT ACCGGT TCCT TTAACCTTAG TAAGGAGG AATTAAAAA
P8 Leader HindIII XbaI
ATGAAAAAGTCTTTAGTCTCAAAGCCTCCGTAGCCGTTGCTACCTCGTTCGGATGCTAAGCTTCGCT TCTAGA
M K K S L V L K A S V A V A T L V P M L S F A S R
NotI HA-tag His-tag Amber stop BglII
GCGGCCGCT TATCCATACGACGTACCGACTACGCA GGAGGT CATCACCATCATCACCAT TAG AGATCT
A A A Y P Y D V P D Y A G G H H H H H * R S
Gene 3 SalI
GGAGGCGGT ACTGTTGAAAAGTTGTTAGCAAAA ---- GCTAACATACCTGCGTAATAAGGAGTCTTAA GTCGAC
G G G T V E S C L A K ---- A N I L R N K E S *

PABMD2 vector: sequence from AgeI to SalI

lac promoter/lac O1 AgeI EP S/D
AATTGTGAGCGGATAACAATT ACCGGT TCCT TTAACCTTAG TAAGGAGG AATTAAAAA
pelB Leader NcoI PstI XbaI
ATGAAATACCTATTGCCCTACGGCAGCCGCTGGATTGTTATTACTCGCGGCCAGCCGCCATGGCGGCCCTGCAGGCCCTCTAGA
M K Y L L P T A A A G L L L L A A Q P A M A A L Q A S R
NotI HA-tag His-tag Amber stop BglII
GCGGCCGCT TATCCATACGACGTACCGACTACGCA GGAGGT CATCACCATCATCACCAT TAG AGATCT
A A A Y P Y D V P D Y A G G H H H H H * R S
Gene 3 SalI
GGAGGCGGT ACTGTTGAAAAGTTGTTAGCAAAA ---- GCTAACATACCTGCGTAATAAGGAGTCTTAA GTCGAC
G G G T V E S C L A K ---- A N I L R N K E S *

Fig. 22B

GR1 Sequence Range: 1 to 146

XbaI	10	20	30	40	50
	<u>TCTAGAGGTGGAGGAGGTGAGGAGAAGTCCCGGCTGTTGGAGAAGGAGAA</u>				
	S	R	G	G	E
	60	70	80	90	100
	CCGTGAACTGGAAAAGATCATCTGCTGAGAAAAGAGGAGCGTGCTCTGAAC				
	R	E	L	E	K
	110	120	130	140	150
	TGCGCCATCAACTCCAGTCTGTAGGAGGTGTTAATAGGGCGCGCC				
	L	R	H	Q	L
	160	170	180	190	200
	* * * * *				

GR2 Sequence Range: 1 to 140

XhoI	10	20	30	40	50
	<u>TCTCGAGGAGGTGGTGAACATCCCGCCTGGAGGGCTACAGTCAGAAAA</u>				
	S	R	G	G	E
	60	70	80	90	100
	CCATCGCCTGCGAATGAAGATCACAGAGCTGGATAAAGACTTGAAGAGG				
	H	R	L	R	M
	110	120	130	140	150
	TCACCATGCAGCTGCAGGACGTGCGAGGTGCGCGCGCGC				
	V	T	M	Q	L
	160	170	180	190	200
	* * * * *				

Fig. 23

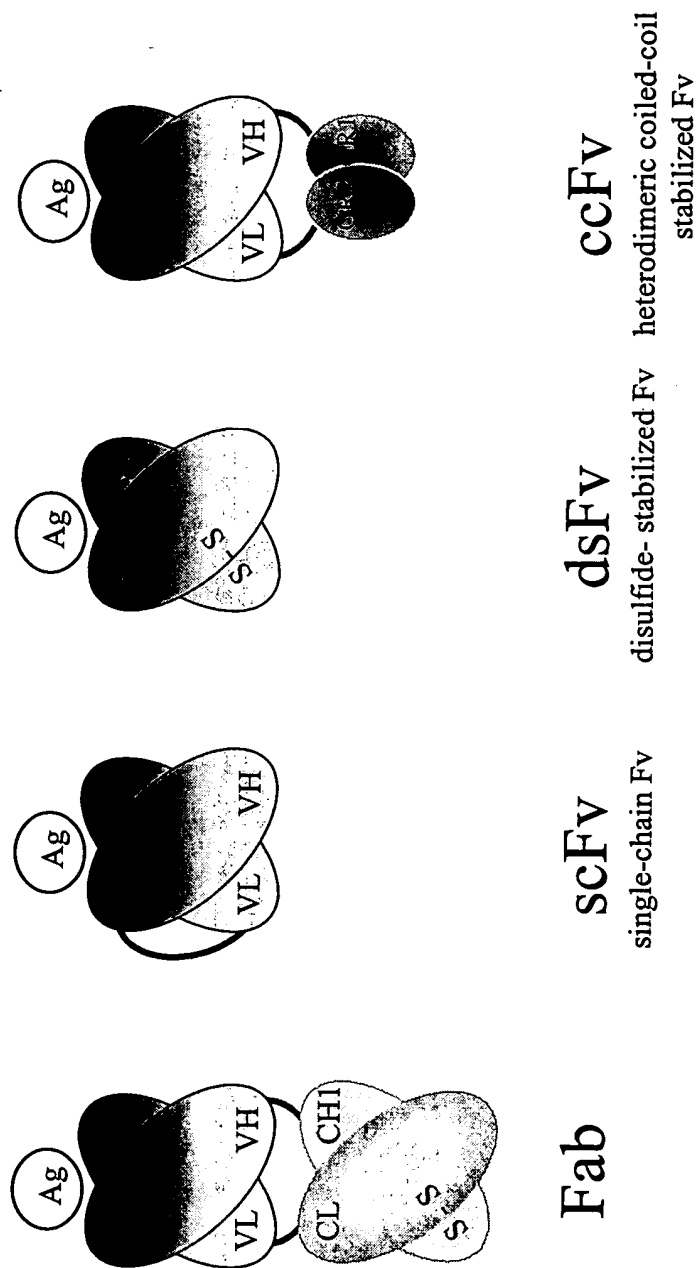


Fig. 24

Expression vector for Adapter-directed bacterial display

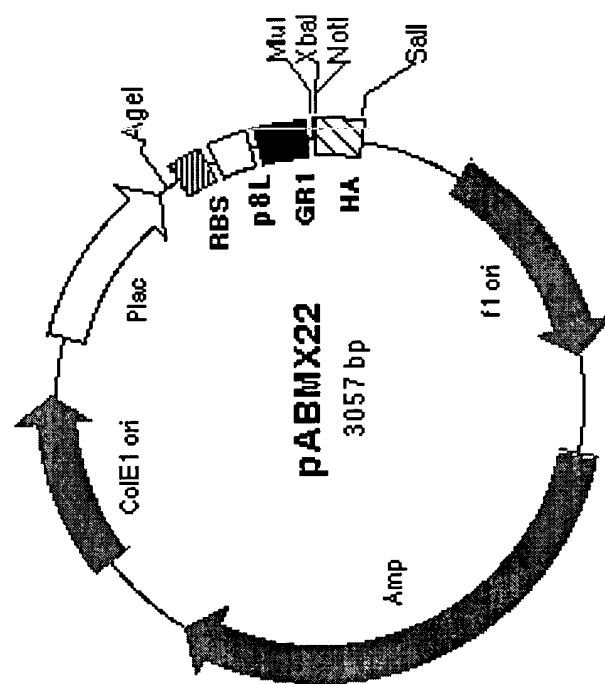


Fig. 25A

Complete vector sequence of pABMX22

GCGCAACGCAATTAATGTAGTGTAGCTCAGTATAGGACACCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGAGGATAACAATTTACCGGTCTTTAAGGAGGAATT
 AAAAAATGAAAAAGTCTTTAGTCTCTCAAGGCTCCGTAGCGTGTGCTACCTCGTTCGATGCTTAAGCTTCGCTGGTGAAGGAAAGTCCCGTCTGCTGGAGAAAGAGAACCGTGAACCTGGAAAAAGATC
 ATTGCTGAGAAAGAGAGGAGCTGTTCTGAACTGCGGCTCAACTGAGCTGTAGGCGTTGACGCGTTCTAGAGCGGCCCTTTACCCGTACGACGCTACCGCATGATAAGTCCGACCTCGA
 CCAATTCGCCCTATAGTGTGCTGTTTACAACTGCTGCGCTGTTTACAACTGCTGAGTGGGAAACCTGCGCTTACCACTTAATCGCTTGCACACATCCCGCTTTCGCCAGCTGGCGT
 AATAGCGAAGAGCCCGCACCGATCGCCCTTCCCAACAGTTGCGGAGCTGAAATGGCGAATGGAGCGGCCCTGTAGCGCGCATTAAGCGCGGGGGTGTGTGTTACCGGACGCTGACCGCTAC
 ACTTGCCAGCGCCCTAGCGCCCGCTCTTTCCTTCTTCCCTTCTTCCGACGTTCCCGGCTTCCCGCTCAAGCTTAATCGGGGCTCCCTTTAGGGTTCCGATTTAGTGTCTTACCGGC
 ACCTCGACCCCAAAAACCTTGATTTAGGTGATGTTTACGCTAGTGGGCCATCGCCCTGATAGCGGTTTTCGCCCTTTCGCTTGAAGTCCGCTTTTAATAGTGGACTCTTGTCCAAACTGGA
 ACAACTCAACCCCTATCTCGGTCTATTTGATTTAAGGATTTTTCGCGATTTTCGCGCTATTGGTTAAAAATGAGCTGATTTAAACAAAATTTAACCGCAATTTTAAACAAATATTAAACGCT
 TACAAATTTAGGTGGCACTTTTCGGGGAATGTGCGGGAACCCCTATTGTTTATTTCTAAATACATCAATCAATAATGATCGCTCATGAGACAAATACCTGATAAAATGCTTCAATAATATTGAAA
 AAGGAAGAGTATGAGTATTCACATTTCCGTGCGCTTATTCCTTTTTCGGGCTTTTTCGCTTCTGCTTTCGCTCAACGAGAACGCTTTTAAAGTTCTGCTATGTGGCGGGTATTATCCCGTATT
 CAGAGTGGGTACATCGAACTGGAATCTCAACGCGGTAAATCCTTGAGGTTTTCGCCCGGAAGACGTTTCCCAATGAGAGCACTTTTAAAGTTCTGCTATGTGGCGGGTATTATCCCGTATT
 GAGCGCGGGCAAGAGCAACTCGGTGCGCGCATACACTATTCTCAGAAATGACTTGTGTGACTCTACCACTGATGAGTGTGCTTACCGGATGGCAATGACAGTAAAGAGAAATTTACAGTGTCTGCCAT
 AACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAAAGATCGGAGGACCGAAAGAGCTAACCGCTTTTTCGCAACATGGGGGATCATGTAACTCGCTTGTATCGTTGGNAACCGGAGCTGA
 ATGAAGCCATACCAACGACGAGCGTGACACACGATGCCGTAGCAATGGCAACAACTTAACTGGCGGAACTACTTACTAGCTTCCGGCAACAAATTAATAGACTGGATGGAG
 GCGGATAAGTTGCAGGACCACTTCTCGCTCGGCCCTTCCGGCTGGCTGTTTATTTGCTGATAAATCTGGAGCCGCTGAGCTGGGTCTCGGGTATCATTTGACAGCACTGGGGCCAGATGGTAAAGCC
 CTCCGATCGTAGTTATCTACAGCGGGAGTCAGGCACTATGGATGAACGAAATAGACAGATCGCTGAGATAGTGGCTCACTGATTAAGCATTTGGTAACTGTACAGCAAGTTTACTCATATA
 TACTTAGATTGATTTAAACCTTCATTTTAAATTTAAAGGATCTAGGTGAAGATCCTTTTGTATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCTCAGACCCCGTAGAA
 AAGATCAAGGATCTTCTTGATCTCTTTCGCGGTAATCTGCTGCTTGCAACAAACCAACCGCTACAGCGTGGTTTGTGTTTCCCGGATCAAGAGCTACCAACTCTTTTCCGAAAGGT
 AACTGGCTTTCAGCAGAGCGGAGATACCAATACTGCTTCTAGTGTAGCGGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCTACATACCTCGCTCTGCTAACTGTACCACTGGCTG
 CTGCCAGTGGCGATAGTGTCTTACCGGTTGACTCAAGACGATAGTTACCGGATAAGCGGCAAGCGGTTCGGCTGAACCGGGGGTTCGTCACACAGCCCACTTGGAGCGAACGACCTACACC
 GAAGTGAATACCTACAGCTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGAGAAAGCGGAGACAGGTATCCGGTAAGCGGCGAGGTTCGGAACAGAGAGCGGAGGAGCTTCCAGGGGAA
 CGCTGGTATCTTTATAGTCTGCGGTTCGCGCTTCCGCTGACTGAGCTCGATTTTGTGATGCTGCTAGGGGGCGGAGCTTATGGAAGAACCGCAACCGCGGCTTTTACGGTTCTCTGG
 CTTTTCGCTGGCCCTTTTCTGCTACATGTTCTTTCCTGCGTTATCCCTGATTTACCGCTTTCGCTGAGTAAACCGGTATTCGCTGAGTAAACCGCTCGCCGCGGACGACCGGAGCGGAGTCA
 GTGAGCGGAGAAAGCGGAGAGCGCCCAATACGCAACCGGCTCTCCCGCGGCTTGGCGGATTCATTAATGCACTGGCACACAGGTTTCCCGACTTGGAAAGCGGGCAGTCA

Fig. 25B

Helper vector for adapter-directed bacterial display

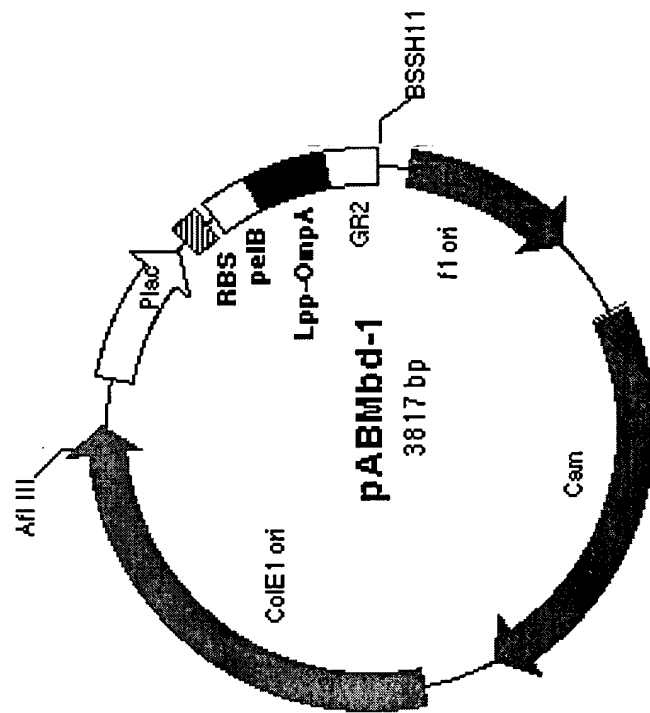


Fig. 26A

